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Effects of Heavy Metal Ions on Microbial Reductive Dechlorination of 1, 2-Dichloroethane and Tetrachloroethene

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Microbial reductive dechlorination has been considered an effective process for the clean-up of organohalide-contaminated sites. Heavy metal ions are commonly present as co-contaminants in various organohalide-contaminated sites. To understand the impacts of heavy metal ions on the environmental fate of organohalides, we investigated the effects of Zn^{2+}, Cu^{2+} and Cd^{2+} on reductive dechlorination of tetrachloroethene (PCE) and 1,2-dichloroethane (1,2-DCA) in sediment microcosms and transferred enrichment cultures. PCE and 1,2-DCA-dechlorinating enrichment cultures could be consecutively transferred in the presence of up to 10 mg/L Cu^{2+} or 10 mg/L Zn^{2+}; by comparison, up to 50 mg/L Cd^{2+} had minor impacts on the microbial reductive dechlorination of PCE and 1,2-DCA. The inhibitory effects of tested heavy metal ions on microbial reductive dechlorination ranked in descending order are Zn^{2+}, Cu^{2+}, and Cd^{2+}. Community profiling and principal component analysis indicate that the concentration and type of contaminants (e.g., heavy metal ions, organohalides) shaped the microbial community structure, an observation similar to a prior report. The enrichment of certain organohalide-respring bacteria (e.g., *Dehalococcoides*, *Dehalogenimonas*) during continuous transfers exposed to heavy metal ions suggests that they are capable of tolerating high concentrations of heavy metal ions. Our findings provide insights into the impacts of heavy metal ions on microbial reductive dechlorination and may be helpful for *in situ* bioremediation at sites contaminated with organohalides and heavy metals.

Keywords: reductive dechlorination, tetrachlorethene, 1,2-dichloroethane, heavy metal ions, *Dehalococcoidia*

INTRODUCTION

Organohalides have been produced on a massive scale for a variety of industrial and agricultural applications such as solvents, pesticides, degreasing agents, and active ingredients (Doherty, 2000; He et al., 2021). Improper handling and uncontrolled discharge have resulted in the accumulation of organohalides in various anoxic environments (e.g., groundwater, sediments). Due to severe toxicity, recalcitrance and tendency to accumulate in lipid-rich organs, many organohalides pose great threats to human health and ecosystem functions (Loganathan and Kannan, 1994; Atashgahi et al., 2018b; Potapowicz et al., 2020; Girones et al., 2021). To mitigate the hazardous impacts of toxic organohalides on natural environments, (bio)degradation and (bio)transformation of anthropogenic

organohalides have been intensively studied in the past few decades (Stroo and Ward, 2010; Stroo et al., 2012; Atashgahi et al., 2016). One of the notable findings is the discovery of specialized anaerobic bacteria that can couple the reductive dechlorination of organohalides with energy conservation (i.e., organohalide respiration) (de Bruin et al., 1992; Mohn and Tiedje, 1992; Adrian and Löffler, 2016). Microbial reductive dechlorination has also been demonstrated as a cost-effective and environmentally friendly method for *in situ* remediations of organohalide contaminants (Ellis et al., 2000; Ferguson and Pietari, 2000; Devlin et al., 2004; Aulenta et al., 2006; He et al., 2021).

Various biogeochemical factors can influence the success of contaminated site clean-up. Organohalides are frequently found to co-exist with other volatile organic compounds (e.g., trihalomethane) (Squillace et al., 2002) and nitrate (Nelson et al., 2002), and these chemicals have been demonstrated of negative effects on microbial reductive dechlorination processes (Kuo and Genthner, 1996; Pardue et al., 1996; Zhang et al., 2012). Other overlooked factors that can affect microbial reductive dechlorination are heavy metals [e.g., lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu)] (Arjoon et al., 2012; Lu et al., 2020). Heavy metals are mainly released to soil and subsurface environments from fertilizers, pesticides, industrial wastewater, and metal mining processes, and often co-mingled with anthropogenic organohalides in groundwater plumes (Wuana and Okieimen, 2011; Zhang et al., 2012; Deng et al., 2018; Wu et al., 2019). Despite that heavy metals (e.g., Ni, Cu, Zn) can serve as enzyme cofactors for various biochemical reactions, many of them often exhibit toxic effects on microbial processes at elevated concentrations (Silver, 1996; Juliastuti et al., 2003; Li and Fang, 2007; Altas, 2009; Rahman and Singh, 2020). Several molecular mechanisms, such as (i) substitutive metalligand binding, (ii) reaction of metal ions with cellular thiols, and (iii) participation of transition metals in Fentontype reactions have been proposed to explain such inhibitory effects (Harrison et al., 2007; Roane et al., 2015; Prabhakaran et al., 2016). Heavy metals also can negatively affect key enzymes involved in microbial biotransformation, resulting in stalled biodegradation of contaminants and extended periods for site clean-up (Kuo and Genthner, 1996). For instance, biodegradation of 3-chlorobenzoate and phenol have been found susceptible to several heavy metal ions (e.g., Cd^{2+}, Cr^{6+}, Cu^{2+}, Hg^{2+}) (Kuo and Genthner, 1996). Inhibition of 2,3,4-trichloroaniline biodegradation at the presence of 10 ~ 200 μg/L Cd^{2+} has also been demonstrated (Pardue et al., 1996).

Organohalide-respiring bacteria (OHRB) are microbial specialists that can utilize organohalides as electron acceptors for energy conservation under anoxic conditions (Atashgahi et al., 2016). OHRB have been frequently detected in various contaminated and pristine environments, and may play indispensable but underestimated roles in the natural attenuation and recycling of organohalides (Atashgahi et al., 2016; Atashgahi et al., 2018a; Yang et al., 2020). For instance, members within OHRB genera *Dehalococcoides*, *Dehalogenimonas* and *Desulfoluna* have been frequently found to inhabit marine environments (e.g., marine intertidal sediments, estuary sediments) (Zanaroli et al., 2015; Peng et al., 2020; Xu et al., 2022). Coastal and marine environments are increasingly contaminated by heavy metals due to a variety of anthropogenic activities (e.g., industrial effluents, oil pollution) (Naser, 2013). How heavy metal ions affect certain marine OHRB is not well elucidated. Recently, Lu et al. investigated the inhibitory effects of heavy metal ions (e.g., Cu^{2+}, Cd^{2+}, Cr^{3+}, Pb^{2+}) on the activities of several OHRB, and found that OHRB in mixed cultures generally had a higher tolerance to the toxicity of heavy metal ions compared to the OHRB in pure cultures. For instance, *Dehalococcoides*-containing microcosms exhibited higher tolerance to heavy metal ions compared to the pure culture of *Dehalococcoides* strain CG1 (Lu et al., 2020). Furthermore, some OHRB (e.g., *Dehalococcoides*) have a relatively higher level of tolerance to metal ions than the non-dechlorinating populations (e.g., fermenters, acetogens, methanogens) in the dechlorinating microbial consortia (Lu et al., 2020), suggesting that the addition of a metal ion (e.g., Pb^{2+}, Cu^{2+}) may be used as a strategy to stimulate and enrich certain OHRB. Nonetheless, the enrichment processes and evolution of microbial communities at the presence of heavy metal ions require further investigation.

In this study, we investigated the impacts of different concentrations of Cd^{2+}, Cu^{2+} and Zn^{2+} on reductive dechlorination of tetrachloroethene (PCE) and 1,2-dichloroethane (1,2-DCA). Enrichment cultures were consecutively transferred to evaluate the effects of heavy metal ions on the evolution of microbial communities and to identify OHRB phylotypes that can tolerate high concentrations of heavy metal ions. Our findings demonstrate that heavy metal ions extended the dechlorination period but increased the relative abundances of several OHRB including *Dehalococcoides* and *Dehalogenimonas*. We also obtained 1,2-DCA- and PCE-dechlorinating consortia that can tolerate as high as 50 mg/L Cd^{2+}. This study expands current understanding of the effects of heavy metal ions on microbial reductive dechlorination with an overarching goal for cost-effective remediation at sites where heavy metals are present as co-contaminants.

MATERIALS AND METHODS

Chemicals

PCE, trichloroethylene (TCE), *cis*-1,2-dichloroethylene (*c*DCE), 1,2-DCA (all ≥99%) were purchased from Macklin Biochemical Co., Ltd (Shanghai, China). Vinyl chloride (VC) and ethene (both ≥99%) were purchased from Dalian Special Gases Co., Ltd (Dalian, China). $CuCl_2{\cdot}2H_2O$, and $ZnCl_2$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). $CdCl_2$ was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China). All other chemicals used in this study were analytical grade or of higher purity.

Microcosm Setup and Enrichment Cultures

Medium preparation and anaerobic cultivation were performed following estabolished protocols (Löffler et al., 2005; Yang et al., 2017a; Yang et al., 2017b). Briefly, reduced, bicarbonate-buffered mineral salts medium was boiling under an atmosphere of N_2 to remove dissolved oxygen, cooled down to room temperature and then dispensed into serum bottles flushed with N_2/CO_2 (80/20, v/v). Sediment samples were collected from the Xi River, Shenyang, Liaoning Province, China (41°44′41″N, 123°17′35″E) as described (Jiang et al., 2022). Microcosms were constructed inside an anoxic chamber (Coy Laboratory Inc., MI, USA) filled with N_2/H_2 (97/3, v/v). An aliquot of 2 mL sediment sludge was pipetted into 160 mL glass serum bottles prefilled with 100 mL of medium mentioned above as described (Yang et al., 2017b; Yang et al., 2020). Bottles were sealed with butyl rubber stoppers (Fushiyuan rubber and plastic products factory, Shenzhen, Guangdong, China) and crimped with aluminum caps (Hongpu Instrument Technology, Ningbo, Zhejiang, China). Lactate (10 mM) was provided as the carbon source and electron donor. A dose of 5 μL neat PCE (*ca.* 0.31 mM or 51.41 mg/L aqueous phase concentration) or 5 μL neat 1,2-DCA (*ca.* 0.62 mM or 61.36 mg/L aqueous phase concentration) was added as the electron acceptor. All bottles were amended with Wolin vitamin mix (Wolin et al., 1963). A heavy metal ion (e.g., Cu^{2+}, Cd^{2+}, Zn^{2+}) was added into each bottle from a stock solution. For each heavy metal ion, three different concentrations (5, 10, and 50 mg/L) were examined. Following the complete dechlorination of PCE or 1,2-DCA to ethene, 1 mL culture suspensison was transferred into fresh medium following the same procedures described above. Additional dechlorinating microcosms and enrichment cultures that did not receive the amenedment of a heavy metal ion were established as controls. The bottles were incubated statically in the dark at 30°C. Microcosms and enrichment cultures were established in duplicate bottles.

16S rRNA Gene Amplicon Sequencing and Bioinformatics

Cells were harvested from 5 mL culture suspension *via* vacuum filtration onto 25 mm diameter 0.22 μm pore-size membrane filters (Merck Millipore Ltd, Darmstadt, Germany). Genomic DNA was extracted from the filters using the Soil DNA Kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions. DNA concentrations were determined using a Qubit 3.0 fluorometer (Invitrogen, Carlsbad, CA, USA). Amplicon sequencing was performed by Suzhou Genewiz Biotechnology (Suzhou, Jiangsu, China). For 16S rRNA gene amplicon sequencing, the primer set Pro341F (5'-CCTACGGRRBGCASCAGKVRVGAAT-3') and Pro806R (5'-GGACTACNVGGGTWTCTAATCC-3') modified by Genewiz for relatively conserved regions within the V3 and V4 hypervariable regions were used to generate amplicons (Fu et al., 2016). The quality and quantity of DNA libraries were evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and a Qubit 2.0 Fluorometer, respectively. DNA libraries were multiplexed and loaded on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) following the manufacturer's instructions. Sequencing was performed using a 2x250 paired-end (PE) configuration and image analysis and base calling were conducted using the MiSeq Control Software (MCS) embedded in the MiSeq instrument. The raw data were processed using Cutadapt (v1.9.1), VSEARCH (v1.9.6), and QIIME (v1.9.1) with default parameters to obtain clean reads (Caporaso et al., 2010; Bhute et al., 2016). Following quality control, sequences were grouped into operational taxonomic units (OTUs) using VSEARCH and QIIME at a 97% sequence identity. All OTUs were assigned to the lowest possible taxonomic rank using RDP classifier 2.2 and Silva_132 16S rRNA database (Wang et al., 2007; Edgar, 2010). Number of the obtained sequences in each sample was between 58,306 and 182,628, and a total of 686 OTUs were clustered from all the samples. Amplicon sequencing reads were deposited into Figshare repository with the following access address: https://doi.org/10.6084/m9.figshare.19212618.v1.

Analytical Methods

Ethene and chlorinated compounds were analyzed using an Agilent 7890B gas chromatography equipped with an Agilent 7697A automatic headspace sampler, a flame ionization detector (FID) (method detection limit ~ 0.2μM) and an Agilent DB-624 capillary column (60 m length x 0.32 mm inner diameter x 1.8 μm film thickness) as described (Yang et al., 2017b). Oven temperature was initially held at 60°C for 2 min, increased to 200°C at 25°C/min, and held at 200°C for 1 min. Inlet and FID temperatures were set at 200°C and 300°C, respectively.

Data Analysis

Principal component analysis (PCA) is a widely used statistical technique for dimension reduction and can be used for resolving differences among samples with distinct treatments. PCA was performed using the PCA plug-in (version 1.50) in OriginPro 2021 (OriginLab Corp. Northampton, MA, USA). Heatmaps of microbial communities were plotted using the ggplot2 packages (Wickham, 2011) embedded in R language (version 4.1.1) (R Core Team, 2020).

RESULTS

Microbial Dechlorination of PCE and 1,2-DCA and Microbial Community Profiles

PCE and 1,2-DCA dechlorinating cultures were enriched from urban river sediments with lactate as the electron donor and carbon source. The enrichment cultures maintained stepwise PCE-to-ethene dechlorination and 1,2-DCA-to-ethene dihaloelimination after two consecutive transfers (**Figure 1A**). In the PCE-dechlorinating enrichment cultures, the initial 46.40 ± 0.72 μmol PCE was dechlorinated to stoichiometric amounts of ethene within 30 days *via* hydrogenolysis with TCE, *c*DCE, and VC being sequentially produced. *Geobacter* and *Dehalococcoides* were the dominant OHRB genera in the second transferred

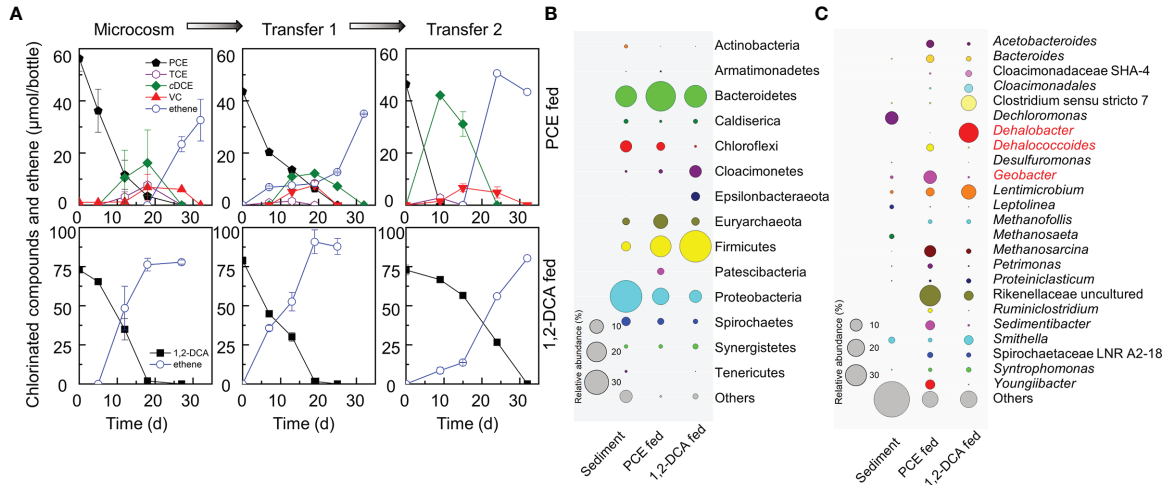

FIGURE 1 | PCE and 1,2-DCA dechlorination by microcosms and transferred enrichment cultures **(A)**, and the microbial structures of the second transferred PCE- and 1,2-DCA-fed enrichment cultures at the phylum level **(B)** and the genus level **(C)**. PCE-fed and 1,2-DCA-fed enrichment cultures serving as the positive controls, maintained PCE-to-ethene stepwise hydrogenolysis and 1,2-DCA-to-ethene dihaloelimination, respectively. Representative microorganisms potentially performing reductive dechlorination are highlighted in red. The sizes of circles represent relative abundances.

enrichment cultures with relative abundances of 11.12% and 3.42%, respectively. *Geobacter lovleyi* strain SZ, isolated from Su-Zi Creek sediment of Korea in 2005, and *Geobacter lovleyi* strain LYY, isolated from black-odorous urban river sediment of China, are capable of PCE-to-*c*DCE dechlorination (Sung et al., 2006; Amos et al., 2007; Liang et al., 2021). This information suggested that the *Geobacter* population in the PCE-dechlorinating enrichment was responsible for the reductive dechlorination of PCE to *c*DCE, which was futher dechlorinated to ethene by *Dehalococcoides*.

In the 1,2-DCA-dechlorinating enrichment cultures, the intial 72.86 ± 3.09 μmol 1,2-DCA was dihaloeliminated to stoichiometric amounts of ethene within 30 days. *Dehalobacter* was the most abundant OHRB genus in the second transfer cultures with a relative abundance of 24.52% (**Figure 1C**), indicating that *Dehalobacter* may be responsible for dihaloelimination of 1,2-DCA. *Dehalobacter* sp. strain WL is able to dechlorinate 1,2-DCA to ethene using a novel reductive dehalogenase (RDase), which shares a 92% similarity to the dihaloeliminating DcaA of *Desulfitobacterium dichloroeliminans* strain DCA1 (Grostern and Edwards, 2006; van der Zaan et al., 2009). To date, the ability to perform dihaloelimination only has been identified in four organohalide-respiring genera including *Dehalobacter*, *Desulfitobacterium*, *Geobacter* and *Dehalogenimonas* (De Wildeman et al., 2003; Grostern and Edwards, 2009; Yan et al., 2009; Jiang et al., 2022).

Differential Effects of Heavy Metal Ions on PCE Dechlorination

To investigate the effects of heavy metal ions on dechlorination processes, sediment microcosms were set up with urban river sediments to dechlorinate PCE or 1,2-DCA at the presence of elevated concentration (i.e., 5, 10 or 50 mg/L) of a heavy metal ion (i.e., Zn^{2+}, Cu^{2+} or Cd^{2+}). In control cultures (i.e., without the addition of heavy metal ions), complete PCE-to-ethene dechlorination was achieved in microcosms and the transferred enrichment cultures within one month (**Figure 1A**). Complete dechlorination of PCE to ethene was also accomplished within one month in sediment microcosms amended with a heavy metal ion (i.e., Zn^{2+}, Cu^{2+} or Cd^{2+}) at a concentration up to 50 mg/L (**Figure 2A**), suggesting that the tolerance of OHRB to heavy metal ions can be greatly enhanced with protective solid matrix (e.g., minerals). Subsequent transfers of the PCE-dechlorinating enrichment cultures at the presence of relatively low concentrations (i.e., 5 or 10 mg/L) of a heavy metal ion were successful (**Figure 2B**), indicating that the enrichment cultures inherited the ability to tolerate the toxicity of Zn^{2+}, Cu^{2+} and Cd^{2+} in low concentrations. By comparison, PCE-to-ethene dechlorination could not be sustained and stalled activity was observed in the first and second transfer cultures when exposed to 50 mg/L Zn^{2+} or 50 mg/L Cu^{2+} during a 80 days' incubation (**Figure 2A**). PCE was completely dechlorinated to ethene in the transferred enrichment cultures exposed to 50 mg/L Zn^{2+} after incubated for about 200 days; nevertheless, complete PCE dechlorination did not occur in transferred enrichment cultures at the presence of 50 mg/L Cu^{2+} after incubated for one and half years (data not shown). In the first transfer, reduced PCE dechlorination rate (i.e., 40 days for complete dechlorination) was observed in enrichment cultures at the presence of 5 or 10 mg/L Zn^{2+}. When the concentration of Zn^{2+} was increased to 50 mg/L, 7.04 ± 1.97 μmol TCE, negligible *c*DCE and VC were detected in the first transfer on day 68 (**Figure 2A**). The inhibitory effects of 50 mg/L Cu^{2+} on the dechlorination of PCE were similar to Zn^{2+} (**Figure 2A**). Unexpectedly, up to 50 mg/L Cd^{2+} did not affect PCE-to-ethene dechlorination activity, and complete

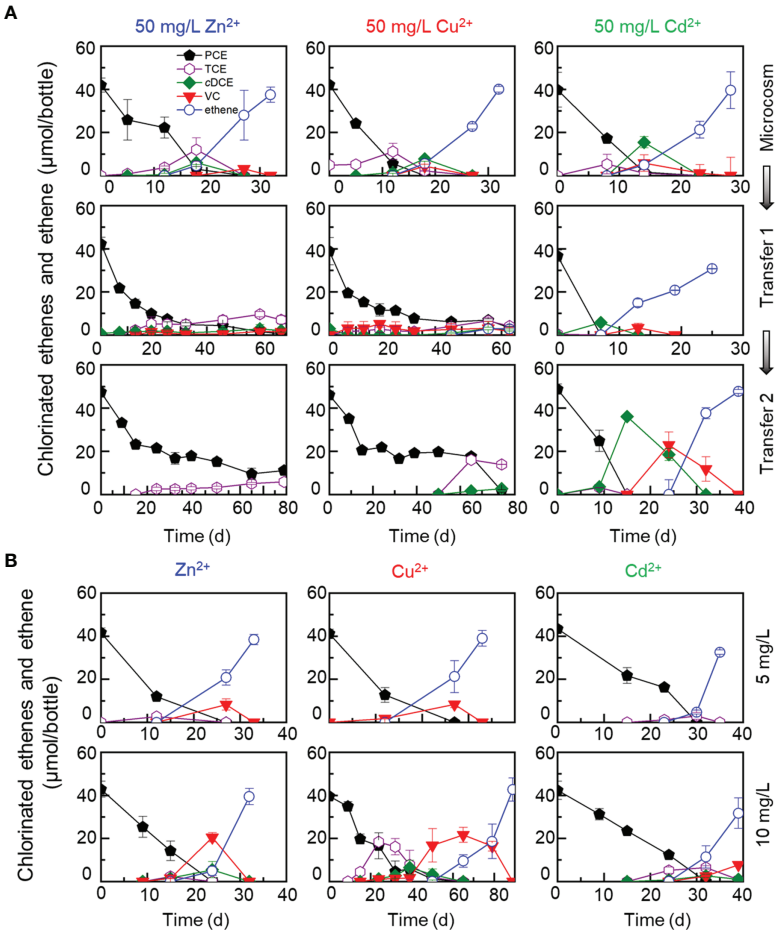

FIGURE 2 | Effects of metal ions (50 mg/L Zn^{2+}, Cu^{2+}, Cd^{2+}) on dechlorination of PCE during transferred enrichment processes **(A)**, and effects of metal ions (5 and 10 mg/L Zn^{2+}, Cu^{2+}, Cd^{2+}) on dechlorination of PCE in the second transferred enrichment cultures **(B)**. High concentrations of Zn^{2+} and Cu^{2+} inhibited the PCE-ethene dechlorination, and no daughter products were detected or detected in small amounts in the transferred enrichment cultures. The decrease of PCE concentrations in transferred enrichment cultures exposed to 50 mg/L Cu^{2+} or Zn^{2+} was partly due to abiotic loss (e.g., adsorption to the butyl rubber stoppers).

PCE dechlorination to stoichiometric amounts of ethene was accomplished within 40 days (**Figures 2A, B**).

Differential Effects of Heavy Metal Ions on 1,2-DCA Dihaloelimination

Without the addition of a heavy metal ion, sediment microcosms and transferred enrichment cultures completely dechlorinated the initial 72.86 ± 3.09 μmol 1,2-DCA to stoichiometric amounts of ethene within 25 days (**Figure 1A**). In sediment microcosms amended with up to 50 mg/L of a heavy metal ion (e.g., Cd^{2+}, Zn^{2+} or Cu^{2+}), 1,2-DCA could also be dihaloeliminated to ethene within 30 days (**Figure 3A**), indicating that these heavy metal ions at a concentration less than 50 mg/L had no apparent inhibitory effects on the reductive dechlorination of 1,2-DCA. Nevertheless, 1,2-DCA-to-ethene dechlorination could not be sustained and was completely lost in the first transfer cultures exposed to 50 mg/L Zn^{2+} and the second transfer cultures exposed to 50 mg/L Cu^{2+} (**Figure 3A**). Unlike the PCE-dechlorinating enrichment cultures, 1,2-DCA was not dechlorinated to ethene after prolonged incubation in the transfer cultures amended with 50 mg/L Zn^{2+} or Cu^{2+} (data not shown). By comparison, up to 10 mg/L Zn^{2+} or Cu^{2+} had little impacts on the dechlorination of 1,2-DCA in the second transfer cultures, and 72.86 ± 3.09 μmol 1,2-DCA was completely dechlorinated to ethene within 30 days (**Figure 3B**). Surprisingly, Cd^{2+} had little inhibitory effects on the dechlorination of 1,2-DCA as well, and 1,2-DCA was completely dechlorinated to ethene within 30 days in microcosms and transfer cultures at the presence of as high as 50 mg/L Cd^{2+} (**Figures 3A, B**).

Impacts of Heavy Metal Ions on Microbial Community Structure

To evaluate effects of heavy metal ions on the dechlorinating communities, 16S rRNA gene amplicon sequencing was applied to samples collected from PCE- or 1,2-DCA-dechlorinating microcosms and transfer cultures exposed to

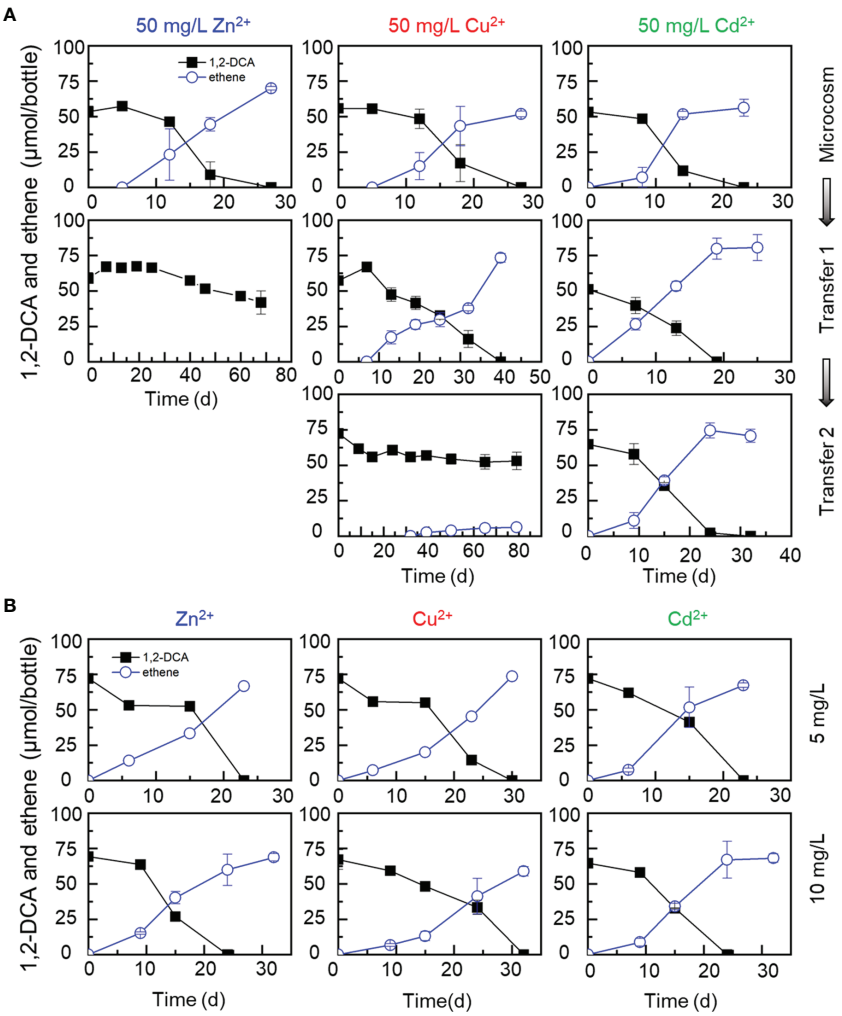

FIGURE 3 | Effects of metal ions (50 mg/L Zn^{2+}, Cu^{2+}, Cd^{2+}) on dihaloelimination of 1,2-DCA during transferred enrichment processes **(A)** and effects of metal ions (5 and 10 mg/L Zn^{2+}, Cu^{2+}, Cd^{2+}) on dechlorination of 1,2-DCA in the second transferred enrichment cultures **(B)**.

different concentrations of heavy metal ions. Proteobacteria, Bacteroidetes, Firmicutes and Chloroflexi were the dominant phyla in sediment microcosms and enrichment cultures. The abundances of Bacteroidetes and Firmicutes increased when PCE or 1,2-DCA was provided (**Figure 1B**). *Geobacter* and *Dehalococcoides* were the main OHRB genera in the second transfer of PCE-dechlorinating cultures where a heavy metal ion was not added. Of note, the relative abundances of *Geobacter* and *Dehalococcoides* were 0.58% and undetectable, respectively, in the river sediment sample (**Figure 1C**). *Dehalobacter* was the most abundant OHRB genus in the second transfer of 1,2-DCA-dechlorinating enrichment cultures without the addition of a heavy metal ion;however, *Dehalobacter* was not detected (i.e., below the detection limit) in the river sediment sample (**Figure 1C**). *Rikenellaceae*, *Lentimicrobium*, *Sedimentibacter*, and *Youngiibacter* were likely the major fermenters in PCE-dechlorinating microcosms that produced acetate and H_2 to supply *Dehalococcoides* and *Geobacter*. By comparison, *Clostridium*, *Lentimicrobium*, *Rikenellaceae*, *Smithella* and *Acetobacteroides* were the most abundant fermenters potentially producing acetate and H_2 for *Dehalobacter* in the 1,2-DCA-dechlorinating enrichment cultures.

Principal component analysis (PCA) demonstrated that the concentrations and types of heavy metal ions and the types of organohalides were the predominant factors shaping the community structures (**Figure 4**). *Geobacter*, *Dehalococcoides*, *Dehalogenimonas*, and *Desulfovibrio* were clustered under the PCE-dechlorinating condition, while *Dehalobacter* was enriched under the 1,2-DCA-dechlorinating condition (**Figure 4A**). Moreover, community structures of the enrichment cultures received an addition of 50 mg/L Cu^{2+} or 50 mg/L Zn^{2+} differed greatly from the enrichment cultures that did not receive the addition of a heavy metal ion and other enrichment cultures, especially the enrichment cultures amended with Cd^{2+} (**Figures 4B, C**).

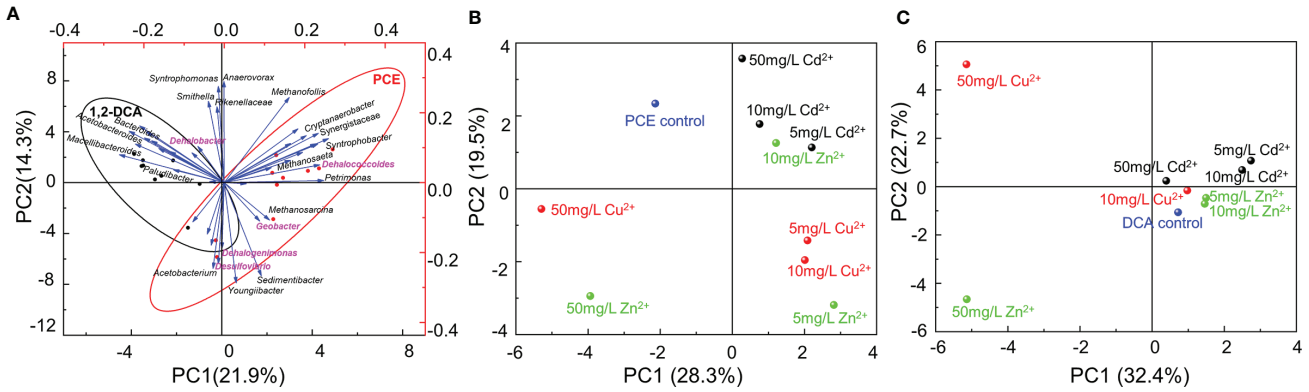

FIGURE 4 | Principal component analysis (PCA) of the microbial communities in PCE- and 1,2-DCA-fed second transferred enrichment cultures amended with gradient concentrations of heavy metal ions: **(A)** PCE- and 1,2-DCA-fed second transferred enrichment cultures; **(B)** PCE-fed second transferred enrichment cultures with different types and concentrations of metal ions; **(C)** 1,2-DCA-fed second transferred enrichment cultures with different types and concentrations of metal ions. Representative microorganisms potentially performing reductive dechlorination are highlighted in purple (Panel **A**).

We also observed that the addition of a heavy metal ion (i.e., Zn^{2+}, Cu^{2+}, Cd^{2+}) likely promoted the enrichment of OHRB, especially the obligate OHRBs (**Figures 5**, **6**). In the second transfer of PCE-dechlorinating enrichment cultures amended with a heavy metal ion, the relative abundances of *Dehalobacter* increased from 0.02% in the control cultures to 4.5% in the enrichment cultures amended with 5 mg/L Zn^{2+}, and 17.2% in the enrichment cultures amended with 10 mg/L Zn^{2+}. *Dehalococcoides*, *Dehalogenimonas*, and *Desulfovibrio* were also enriched with the addition of a heavy metal ion. For instance, the relative abundances of *Dehalogenimonas*, of which certain member is capable of dechlorinating TCE to ethene (Yang et al., 2017b), increased to 48.1% in the enrichment cultures exposed to 50 mg/L Zn^{2+} from 0.1% in the control cultures. Likewise, the relative abundances of *Dehalococcoides* was 11.2% in the enrichment cultures exposed to 10 mg/L Cu^{2+}, compared with 3.5% in control cultures and 5.7% in cultures amended with 5 mg/L Cu^{2+}. In contrast, decreasing abundances of *Geobacter* was found in the enrichment cultures exposed to a heavy metal ion during consective transfers. The relative abundances of *Geobacter* decreased from 11.1% in the control cultures to 6.2% in the enrichment cultures exposed to 50 mg/L Cd^{2+}. The fermenter *Lentimicrobium* was seemingly tolerant to heavy metal ions, and the relative abundances were 4.5%, 8.9% and 15.7% in control cultures, the second transfer cultures exposed to 10 mg/L Zn^{2+} and 10 mg/L Cd^{2+}, respectively.

In the 1,2-DCA-dechlorinating cultures, *Dehalococcoides* and *Desulfovibrio* were greatly enriched in Cu^{2+} amended enrichment cultures. The relative abundance of *Dehalococcoides* in the second transfer of 1,2-DCA-dechlorinating enrichment cultures amended with 50 mg/L Cu^{2+} was 5.7% compared with 0.3% in the control cultures. The relative abundance of *Desulfovibrio* in the second transfer of 1,2-DCA-dechlorinating enrichment cultures amended with 10 mg/L Cu^{2+} was 8.7%, compared with 0.2% in the control cultures. Similar to the PCE-dechlorinating enrichment cultures, *Lentimicrobium* was enriched in 1,2-DCA-dechlorinating enrichment cultures exposed to a heavy metal ion, and the relative abundances were 14.0%, 18.3%, 18.5% and 20.8% in control cultures, the second transfer of enrichment cultures exposed to 10 mg/L Zn^{2+}, 10 mg/L Cu^{2+} and 10 mg/L Cd^{2+}, respectively.

DISCUSSION

In the PCE-dechlorinating enrichment cultures, *Geobacter* and *Dehalococcoides* may play key roles for the sequential dechlorination of PCE to ethene. Cross-feeding interactions between *Geobacter* and *Dehalococcoides*, not limited to electron acceptor dependency, have been frequently observed and investigated. For instance, *Geobacter lovleyi* strain SZ can fulfill the cobamide requirement of *Dehalococcoides* strains (Yan et al., 2012). *Geobacter* can also supply carbon source to *Dehalococcoides* strains by fermenting lactate to acetate (Liang et al., 2021). Also worth mentioning is that, without dynamic monitoring, the abundance and function of *Geobacter* may be underestimated since community structures were only analyzed at the completion of reductive dechlorination. To date, *Geobacter* isolates capable of respiring chlorinated ethenes only couple growth with PCE-to-*c*DCE dechlorination but inactive during the stage of *c*DCE-to-ethene dechlorination, probably due to the depletion of an utilizable electron acceptor (e.g., PCE, TCE) (Lu et al., 2021).

Concerns have been raised over the global contamination of heavy metals and organohalides. An improved understanding of how heavy metal ions can impact OHRB and the associated dechlorination processes is critical for the successful bioremediation at the contaminated sites. Our study revealed that the types (i.e., Zn^{2+}, Cu^{2+}, Cd^{2+}) and concentrations of heavy metal ions posed differential effects on the microbial dechlorination of PCE and 1,2-DCA. A previous microcosm study showed minor inhibitory effects of heavy metal ions (i.e., Cd^{2+}, Cr^{3+}, Pb^{2+}) at concentrations less than 20 mg/L on the PCE dechlorination in sediment microcosms (Lu et al.,

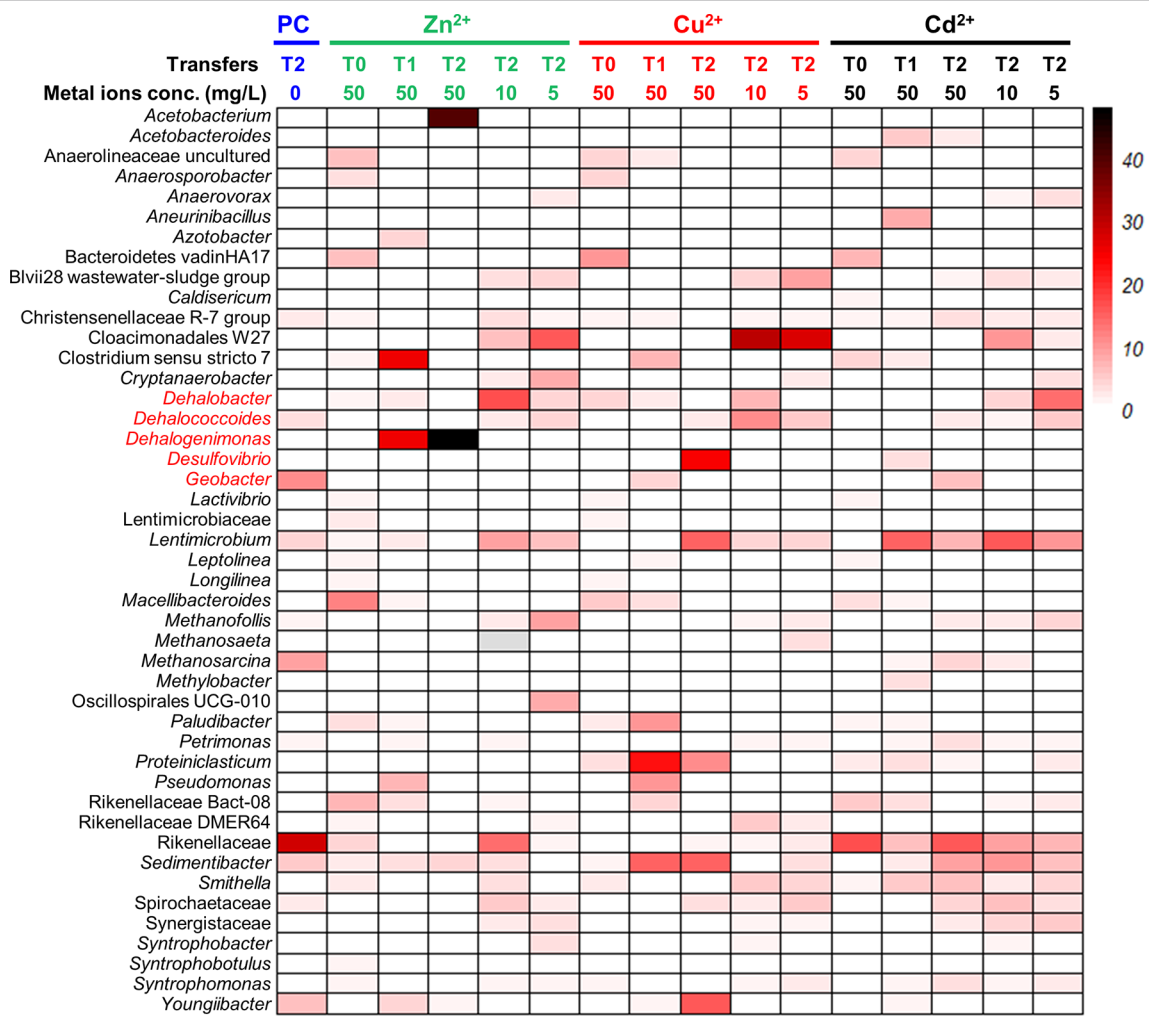

FIGURE 5 | Microbial communities profiling of PCE-fed enrichment cultures amended with gradient concentrations of heavy metal ions. PC represents biotic controls without heavy metal amendments. T0: sediment microcosms; T1: the first transferred enrichment cultures; T2: the second transferred enrichment cultures. The numbers below the transfers represent the concentrations of heavy metal ions with the unit "mg/L".

2020), which was not completely in line with the observation in this study. Such inhibitory effects of heavy metal ions on the dechlorination processes have been reported (Said and Lewis, 1991; Pardue et al., 1996; Kong, 1998). The inconsistency between our study and previous studies may be caused by differential geochemical and microbial compositions in source materials (e.g., sediments) used for microcosm construction. Therefore, conclusions regarding the tolerance of dechlorinating microcosms or pure cultures should be evaluated with precaution and a number of factors (e.g., minerals, pH, salinity, ionic strength) should be considered.

Cd^{2+} is detrimental to various biological functions, such as competition with Zn^{2+} for the active sites of proteins and enzymes, interference with calcium metabolism, and covalently binding to the -SH group (Hall, 2002). The inhibitive concentration of Cd^{2+} on trichloroaniline degradation could be as low as 0.01 mg/L in a mineral-dominated soil (Pardue et al., 1996). Previous studies suggested that Cd^{2+} had inhibitory effects on 1,2-DCA degradation and the presence of 33.7 mg/L Cd^{2+} resulted in partial 1,2-DCA degradation (Arjoon et al., 2015). Recently, another study also demonstrated that Cd^{2+} was one of the most toxic heavy metal ions that inhibited the PCE-to-ethene dechlorination process in pure cultures, mixed cultures, and microcosms (Lu et al., 2020). Unexpectedly, we did not observe significant inhibition of up to 50 mg/L Cd^{2+} on the dechlorination of PCE or 1,2-DCA in the microcosms and enrichment cultures. Such discrepancy and, especially, the mechanisms behind the tolerance of organohalide-respiring *Dehalococcoidia* to Cd^{2+}requires further investigation.

Certain OHRB can have a relatively higher tolerance to heavy metal ions, and the relative abundances of OHRB in the microbial community increases when exposed to a heavy metal ion. For instance, 50 mg/L Zn^{2+} may promote the growth of *Dehalogenimonas* (PCE-dechlorinating cultures), 50 mg/L Cu^{2+} may be conducive to the enrichment of *Dehalococcoides* (PCE- and 1,2-DCA-dechlorinating

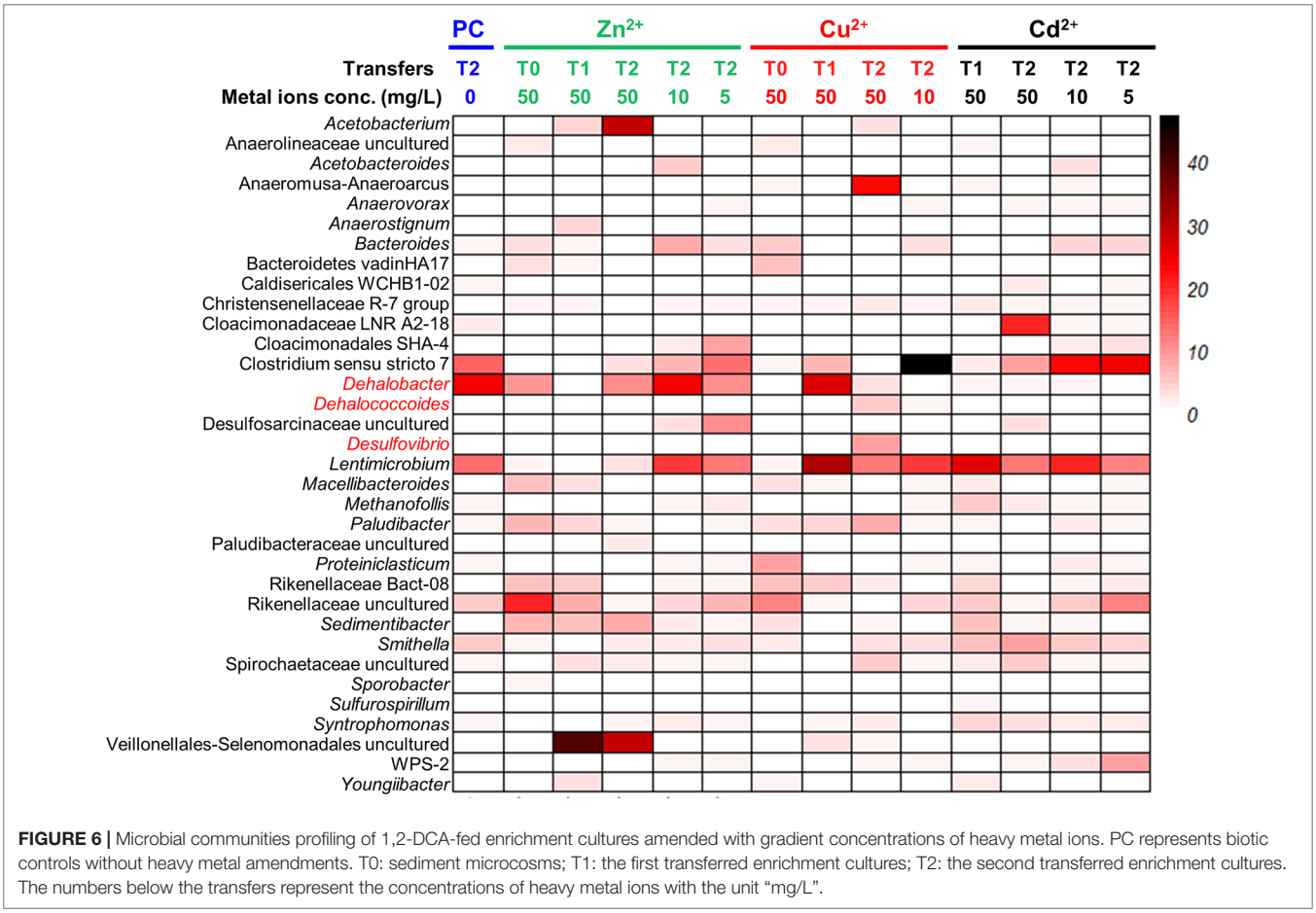

FIGURE 6 | Microbial communities profiling of 1,2-DCA-fed enrichment cultures amended with gradient concentrations of heavy metal ions. PC represents biotic controls without heavy metal amendments. T0: sediment microcosms; T1: the first transferred enrichment cultures; T2: the second transferred enrichment cultures. The numbers below the transfers represent the concentrations of heavy metal ions with the unit "mg/L".

cultures) and *Dehalobacter* (1,2-DCA-dechlorinating cultures), and 50 mg/L Cd^{2+} may enhance the growth of *Geobacter* (PCE-dechlorinating cultures). In the meantime, non-dechlorinating populations such as *Acetobacterium*, *Sedimentibacter*, *Rikenellaceae*, *Lentimicrobium*, *Youngiibacter* in the PCE-dechlorinating cultures, and *Acetobacterium* and *Lentimicrobium* in the 1,2-DCA-dechlorinating cultures were also enriched when exposed to a heavy metal ion. How heavy metal ions affect the interaction of syntrophic metabolic networks involving OHRB and other beneficial populations (e.g., *Desulfovibrio*, *Acetobacterium*, *Methanosarcina*) for interspecies carbon, electron, and cobamide transfers (Men et al., 2014; Wang et al., 2019; Lu et al., 2020) is still largely unknown. Synthetic and systematic studies using omics tools may assist us in unraveling the syntrophy mechanisms.

In summary, our study demonstrated that: (1) the inhibitions of heavy metal ions on PCE and 1,2-DCA reductive dechlorination depend on the concentrations and types of the heavy metal ions, an observation similar to a previous study (Lu et al., 2020); (2) certain OHRB had a relatively high tolerance to heavy metal ions, of which Zn^{2+} may be the most toxic heavy metal ion to reductive dechlorination; and (3) the 1,2-DCA- and PCE-dechlorinating enrichment cultures can tolerate as high as 50 mg/L Cd^{2+}.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://figshare.com/, doi.org/10.6084/m9.figshare.19212618.v1.

AUTHOR CONTRIBUTIONS

JW, XL, JY, and YY contributed to the conception and design of the study. JW and XL performed the experiments and data analysis. JW and YY wrote the first draft of the manuscript. . All authors contributed to manuscript revision, read, and approved the submitted version.

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